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Enhancement of mucosal immune responses to the influenza virus HA protein by alternative approaches to DNA immunization.

Sha Z, Vincent MJ, Compans RW.

Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA.

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DNA immunization provides many advantages as an approach to prevent infectious diseases. However, although previous studies using this approach have demonstrated immune responses in serum, they were not successful in inducing significant levels of antibodies in secretions. In this study, plasmid DNAs expressing the influenza virus hemagglutinin glycoprotein have been evaluated for their ability to induce antibody responses in serum and saliva when used alone or along with either liposomes or bioadhesive polymers as mucosal delivery vehicles. Significant levels of virus-specific Ig in serum as well as secretory IgA in saliva were detected in mice following mucosal DNA immunization. These antibodies were found to block the infectivity of the virus using a plaque reduction assay. Our findings thus indicate that mucosal DNA immunization with specific delivery systems can elicit virus-specific antibody responses in serum as well as IgA responses at mucosal surfaces.

PMID: 10084693 [PubMed - indexed for MEDLINE]

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Enhanced protection against a lethal influenza virus challenge by immunization with both hemagglutinin- and neuraminidase-expressing DNAs.

Chen Z, Matsuo K, Asanuma H, Takahashi H, Iwasaki T, Suzuki Y, Aizawa C, Kurata T, Tamura S.

Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan.

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The ability of plasmid DNA encoding hemagglutinin (HA), neuraminidase (NA) or matrix protein (M1) from influenza virus A/PR/8/34 (PR8) (H1N1), and mixtures of these plasmid DNAs (HA + NA and HA + NA + M1) to protect against homologous or heterologous virus infection was examined in BALB/c mice. Each DNA was inoculated twice, 3 weeks apart, or four times, 2 weeks apart, at a dose of 1 microg of each component per mouse by particle-mediated DNA transfer to the epidermis (gene gun). Seven days after the last immunization, mice were challenged with a lethal homologous or heterologous virus and the ability of each DNA to protect the mice from influenza was evaluated by observing lung virus titers and survival rates. The administration of a plasmid DNA mixture of either (HA + NA) or (HA + NA + M1) provided almost complete protection against the PR8 virus challenge, and this protection was accompanied by high levels of specific antibody responses to the respective components. The degree of protection afforded in these groups is significantly higher than that in mice given either HA- or NA-expressing DNA alone, which provided only a partial protection against PR8 challenge or that in mice given M1-expressing DNA, which failed to provide any protection. In addition, both of the plasmid DNA mixtures (HA + NA) and (HA + NA + M1) showed a slight tendency to provide cross-protection against an A/Yamagata/120/86 (H1N1) virus challenge, and this was accompanied by a relatively high level of cross-reacting antibodies. Thus, there was no clear difference between the ability of the HA + NA and HA + NA + M1 plasmid DNA mixtures in providing protection against either a PR8 or heterologous virus challenge. These results suggest that in mice immunized by gene gun, a mixture of plasmid DNAs encoding HA and NA can provide the most effective protection against the virus challenge. The addition of the M1-expressing plasmid DNA to this mixture does not enhance the degree of protection afforded.

PMID: 10067670 [PubMed - indexed for MEDLINE]



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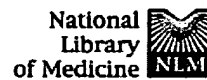
A novel influenza subunit vaccine composed of liposome-encapsulated haemagglutinin/neuraminidase and IL-2 or GM-CSF. I. Vaccine characterization and efficacy studies in mice.

Babai I, Samira S, Barenholz Y, Zakay-Rones Z, Kedar E.

The Lautenberg Center for General and Tumor Immunology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

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The aim of this study was to improve the potency of the currently used influenza subunit vaccines, which are of relatively low efficiency in high-risk groups. Influenza A virus (Shangdong/9/93) haemagglutinin/neuraminidase (H3N2), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2) were encapsulated, each separately or combined, in multilamellar vesicles composed of dimyristoyl phosphatidylcholine. BALB/c mice were immunized once, i.p. or s.c., with 0.05-2.0 microg HN administered either as free antigen (F-HN), adsorbed to aluminum hydroxide (Al-HN), or encapsulated in liposomes (Lip-HN), separately or together with $1 \times 10(2)$ - $4.5 \times 10(4)$ units of free or encapsulated cytokines. Serum antibodies were assayed on days 11-360 by the haemagglutination-inhibition (HI) test and ELISA. Protective immunity against intranasal virus challenge was determined at 9-14 months post-vaccination. The following results were obtained: (1) The efficiency of encapsulation in liposomes was 95, 90 and 38% for HN, IL-2 and GM-CSF, respectively, and the liposomal preparations were highly stable as an aqueous dispersion for > 2 months at 4 degrees C. (2) Following immunization with 0.5 microg Lip-HN, there was an earlier, up to 50-fold stronger, and 3-5 times longer response than that obtained with nonliposomal HN. (3) Coimmunization with free cytokines further increased the response 2-20 times and the two cytokines had an additive effect. (4) Liposomal cytokines were 2-20 times more effective than the free cytokines and their stimulatory effect was more durable. (5) A 100% seroconversion (HI titer > or = 40) was achieved with only 10-25% of the routinely used antigen dose, by encapsulating either antigen or cytokine. (6) The level of protection following vaccination with the combined liposomal vaccines was 70-100% versus 0-25% in mice immunized with Al-HN alone, and no toxicity was observed. In conclusion, our animal experiments show that the liposomal vaccines are superior to the currently used influenza vaccines, increasing the response by 2-3 orders of magnitude in mice. This approach may also prove valuable for subunit vaccines against other microorganisms.



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Chimeric plant virus particles as immunogens for inducing murine and human immune responses against human immunodeficiency virus type 1.

Marusic C, Rizza P, Lattanzi L, Mancini C, Spada M, Belardelli F, Benvenuto E, Capone I.

Divisione Biotecnologie e Agricoltura, ENEA, 00060 Rome, Italy.

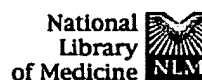
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The high-yield expression of a neutralizing epitope from human immunodeficiency virus type 1 (HIV-1) on the surface of a plant virus and its immunogenicity are presented. The highly conserved ELDKWA epitope from glycoprotein (gp) 41 was expressed as an N-terminal translational fusion with the potato virus X (PVX) coat protein. The resulting chimeric virus particles (CVPs), purified and used to immunize mice intraperitoneally or intranasally, were able to elicit high levels of HIV-1-specific immunoglobulin G (IgG) and IgA antibodies. Furthermore, the human immune response to CVPs was studied with severe combined immunodeficient mice reconstituted with human peripheral blood lymphocytes (hu-PBL-SCID). hu-PBL-SCID mice immunized with CVP-pulsed autologous dendritic cells were able to mount a specific human primary antibody response against the gp41-derived epitope. Notably, sera from both normal and hu-PBL-SCID mice showed an anti-HIV-1-neutralizing activity. Thus, PVX-based CVPs carrying neutralizing epitopes can offer novel perspectives for the development of effective vaccines against HIV and, more generally, for the design of new vaccination strategies in humans.

PMID: 11507188 [PubMed - indexed for MEDLINE]

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☐ 1: Vaccine 1999 Jul 16;17(22):2918-26Related Articles, [NEW Books](#), [LinkOut](#)**ELSEVIER SCIENCE
FULL-TEXT ARTICLE****Mutants of cholera toxin as an effective and safe adjuvant for nasal influenza vaccine.****Hagiwara Y, Komase K, Chen Z, Matsuo K, Suzuki Y, Aizawa C, Kurata T, Tamura S.**

Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan.

The effectiveness and safety of mutants of cholera toxin (CT) as an adjuvant for nasal influenza vaccine was examined. Four CT mutants, called CT7 K (Arg to Lys), CT61F (Arg to Phe), CT112 K (Glu to Lys), and CT118E (Glu to Gln), were produced by the replacement of one amino acid at the A1-subunit using site-directed mutagenesis. All these mutants were confirmed to be less toxic than native CT when the toxicity was analysed by using Y1 adrenal cells in vitro. When high (1 microg) and low (0.1 microg) doses of these CT mutants, together with high (1 microg) and low (0.1 microg) doses of influenza A/PR/8/34 virus (H1N1) vaccine, respectively, were administered intranasally into BALB/c mice in a two dose regimen (twice, 4 weeks apart), they produced both anti-PR8 hemagglutinin (HA) IgA and IgG antibody (Ab) responses roughly in a dose-dependent manner. The relatively low level of anti-HA Ab responses, induced by the low dose CT mutants, were enough to provide complete protection against the homologous virus infection. Under these vaccination conditions, no anti-CTB IgE Ab responses were induced. The mutant CT112 K, which showed a relatively high adjuvant activity, the lowest toxicity and relatively high yields in a bacterial culture, seems to be the most effective and safest adjuvant for nasal influenza vaccine among those examined. The low dose of CT derivatives or vaccine used in the mouse model (0.1 microg/20 g mouse) corresponded to 100 microg/20 kg, the estimated dose per person. A tentative plan for safety standards for human use of CT (or LT) derivatives as an adjuvant of nasal influenza vaccine is discussed.

PMID: 10438064 [PubMed - indexed for MEDLINE]

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